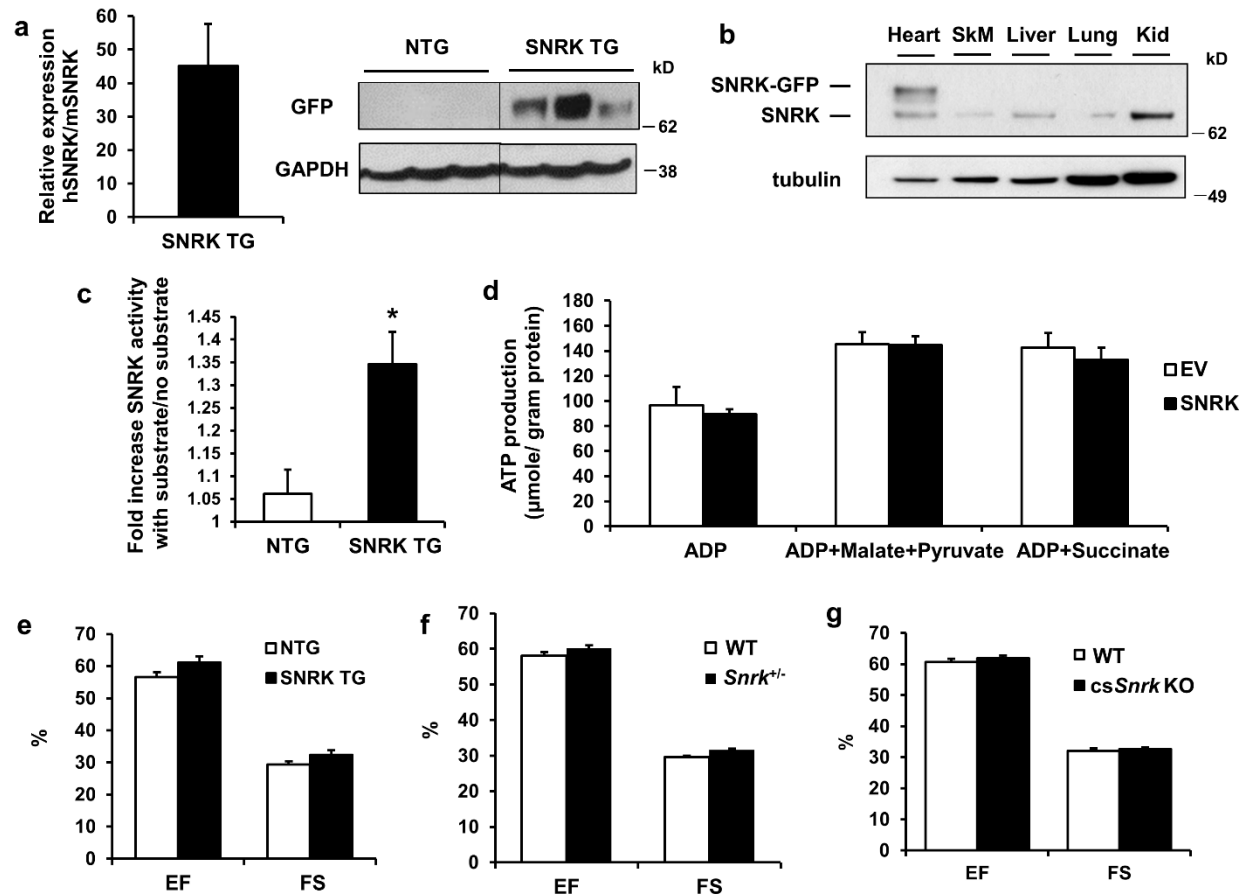
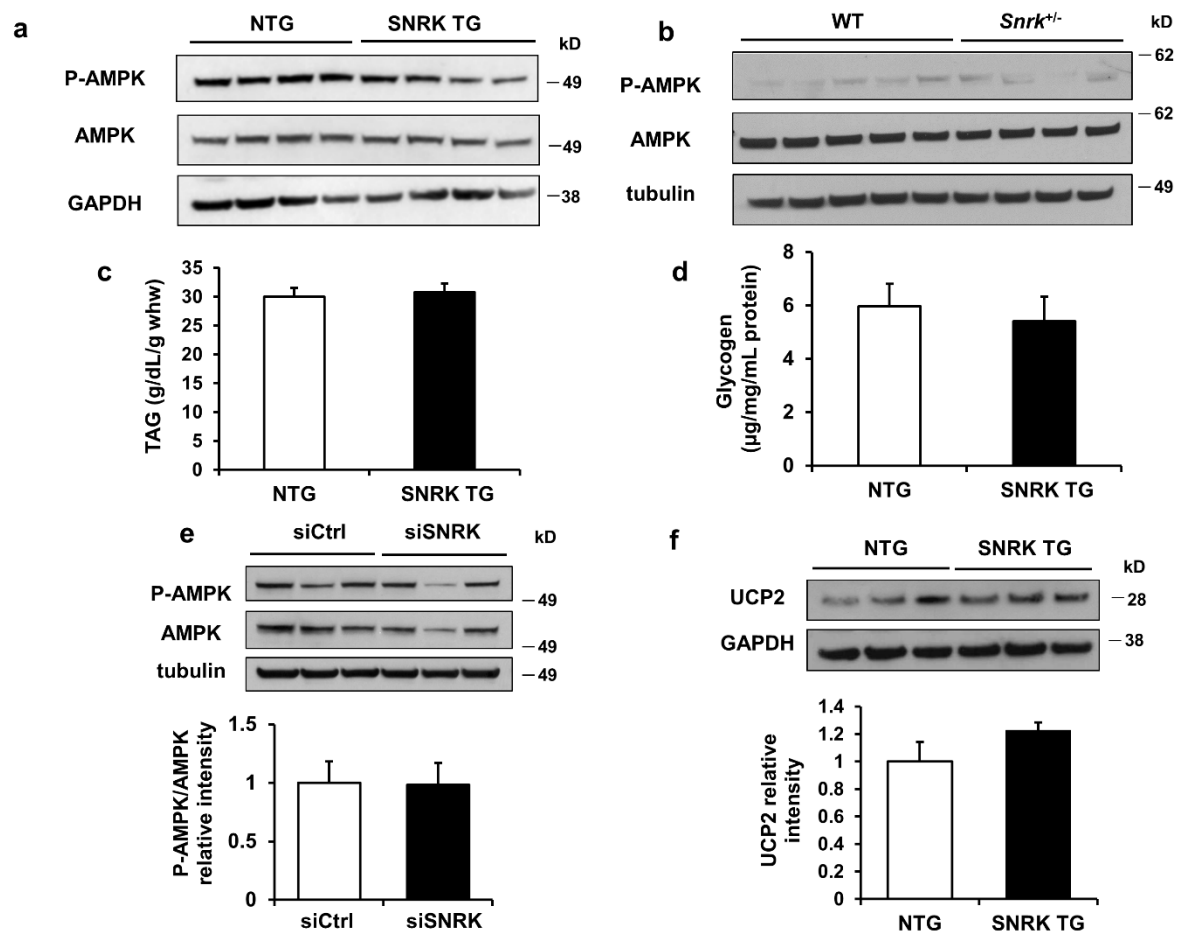


Supplementary Figure 1



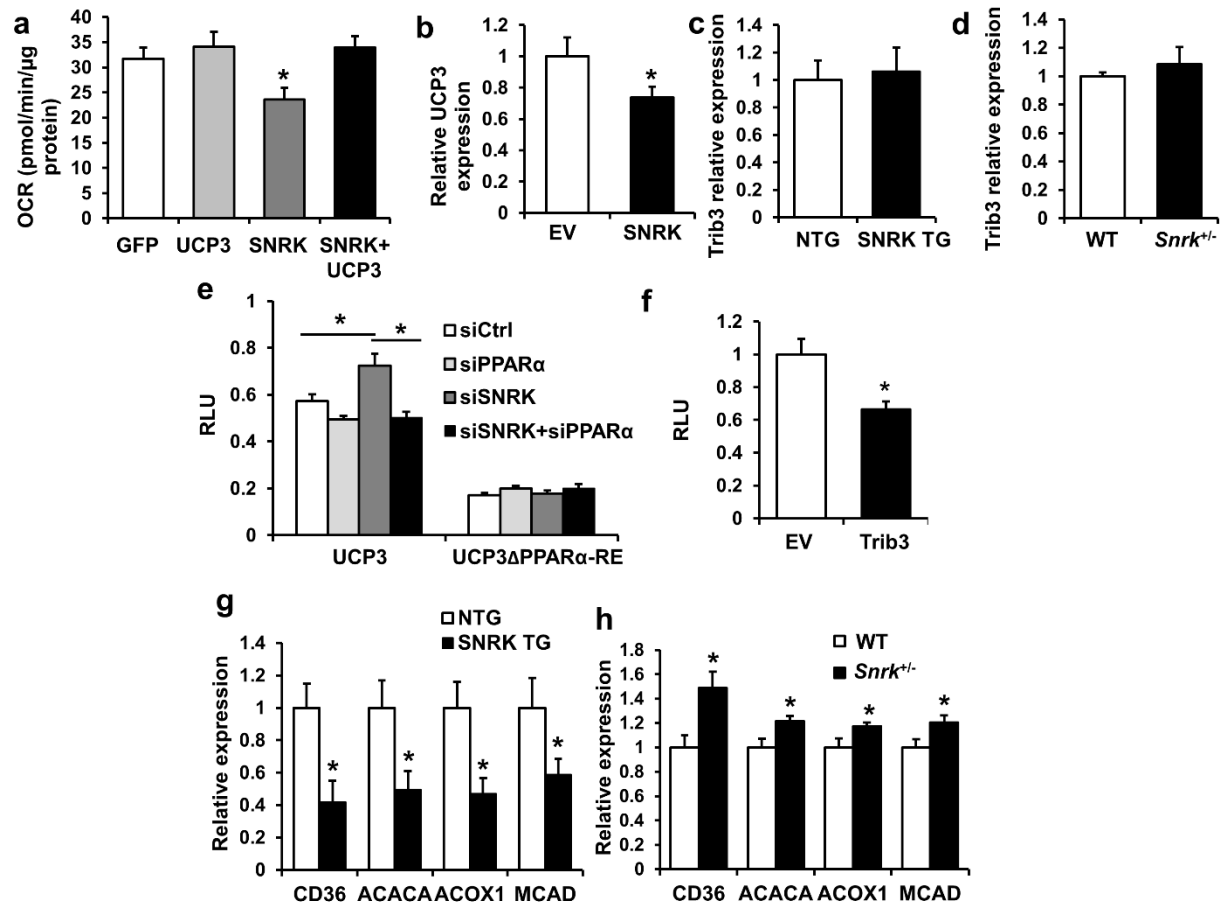
Supplementary Figure 1: SNRK expression and activity in SNRK TG mice, and cardiac performance in SNRK TG, *Snrk*^{+/-}, and *csSnrk* KO mice. (a) mRNA expression of the human SNRK transgene relative to the endogenous mouse SNRK in SNRK TG mice. n=5. Western blot shows SNRK-GFP expression in SNRK TG hearts with GFP antibody. (b) Protein expression of endogenous SNRK, transgenic SNRK-GFP (with SNRK antibody), and tubulin in the heart, skeletal muscle (SkM), liver, lung, and kidney (Kid) in a SNRK TG mouse. (c) SNRK activity as measured in anti-GFP immunoprecipitates in NTG and SNRK TG hearts with H3.3 substrate and normalized to background signal without H3.3 substrate. n=4. (d) ATP production in HL1 cardiomyocytes with empty vector (EV) or SNRK overexpression. ATP was measured in the presence of substrates indicated. n=16. (e) Cardiac ejection fraction (EF) and fractional shortening (FS) in NTG and SNRK TG mice. n=16-18. (f) Cardiac EF and FS in WT and *Snrk*^{+/-} mice. n=3. (g) Cardiac EF and FS in WT and *csSnrk* KO mice. Data are represented as mean ± SEM. **P*≤0.05 by Student's t-test.

Supplementary Figure 2



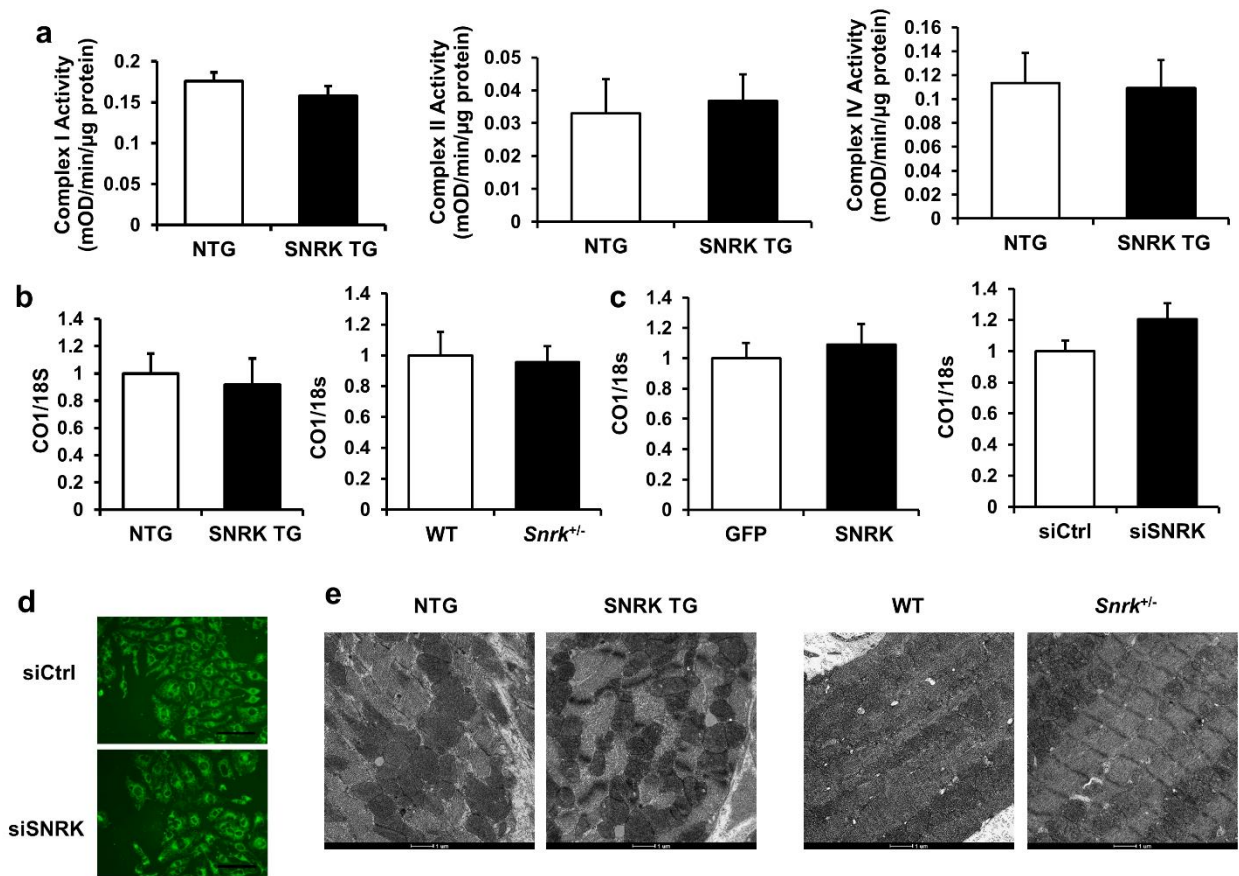
Supplementary Figure 2: SNRK TG mice do not have increased levels of P-AMPK or UCP2, nor depleted triglyceride or glycogen stores. (a) P-AMPK protein levels in NTG and SNRK TG hearts. (b) P-AMPK protein levels in WT and *Snrk*^{+/-} hearts. Triglyceride (TAG, c) and glycogen content (d) in NTG and SNRK TG hearts. n=6. (e) P-AMPK protein levels in HL1 cells with control or SNRK siRNA treatment. (f) UCP2 protein levels in NTG and SNRK TG hearts. Data are represented as mean ± SEM.

Supplementary Figure 3



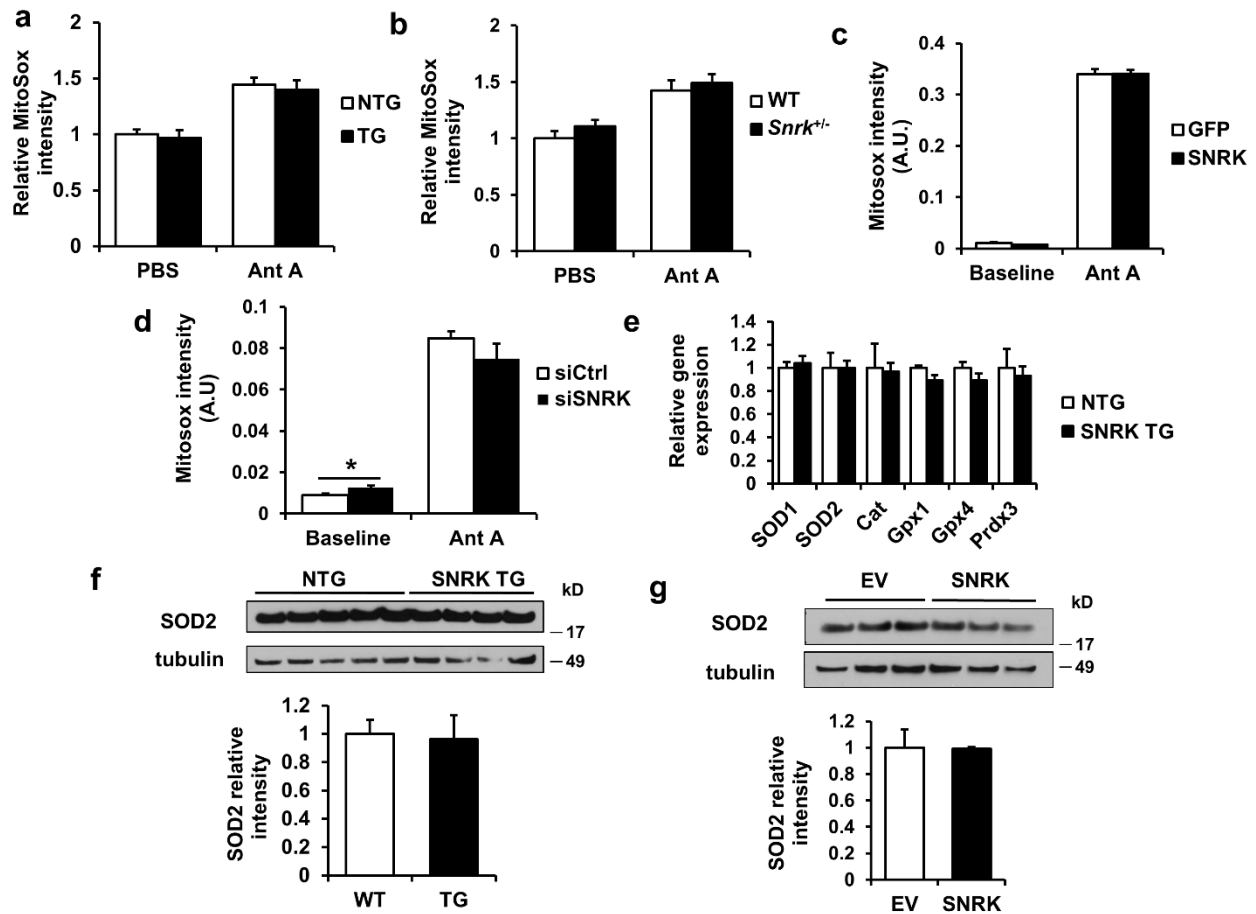
Supplementary Figure 3: SNRK decreases oxygen consumption in a UCP3-dependent manner and increases expression of the UCP3 promoter through PPARα. (a) Oxygen consumption in HL1 cells with overexpression of GFP, UCP3, SNRK, or SNRK+UCP3. n=3. (b) UCP3 mRNA levels with SNRK overexpression in HL1 cells. n=3. Trib3 mRNA levels in (c) NTG and SNRK TG hearts, n=4-5, and (d) WT and SNRK^{+/-} hearts, n=3. (e) Luciferase activity from a UCP3 promoter-luciferase construct and a construct with a PPARα-response element deleted (UCP3ΔPPARα-RE) with SNRK and/or PPARα knockdown in mouse embryonic fibroblasts. n=3. (f) Luciferase activity from a PPARα promoter-luciferase construct with overexpression of an empty vector (EV) or Trib3. n=3. (g) PPARα target gene expression in NTG and SNRK TG hearts. n≥3. (h) PPARα target gene expression in WT and *Snrk*^{+/-} hearts. n≥3. Data are represented as mean ± SEM. *P≤0.05 by one-way ANOVA or Student's t-test.

Supplementary Figure 4



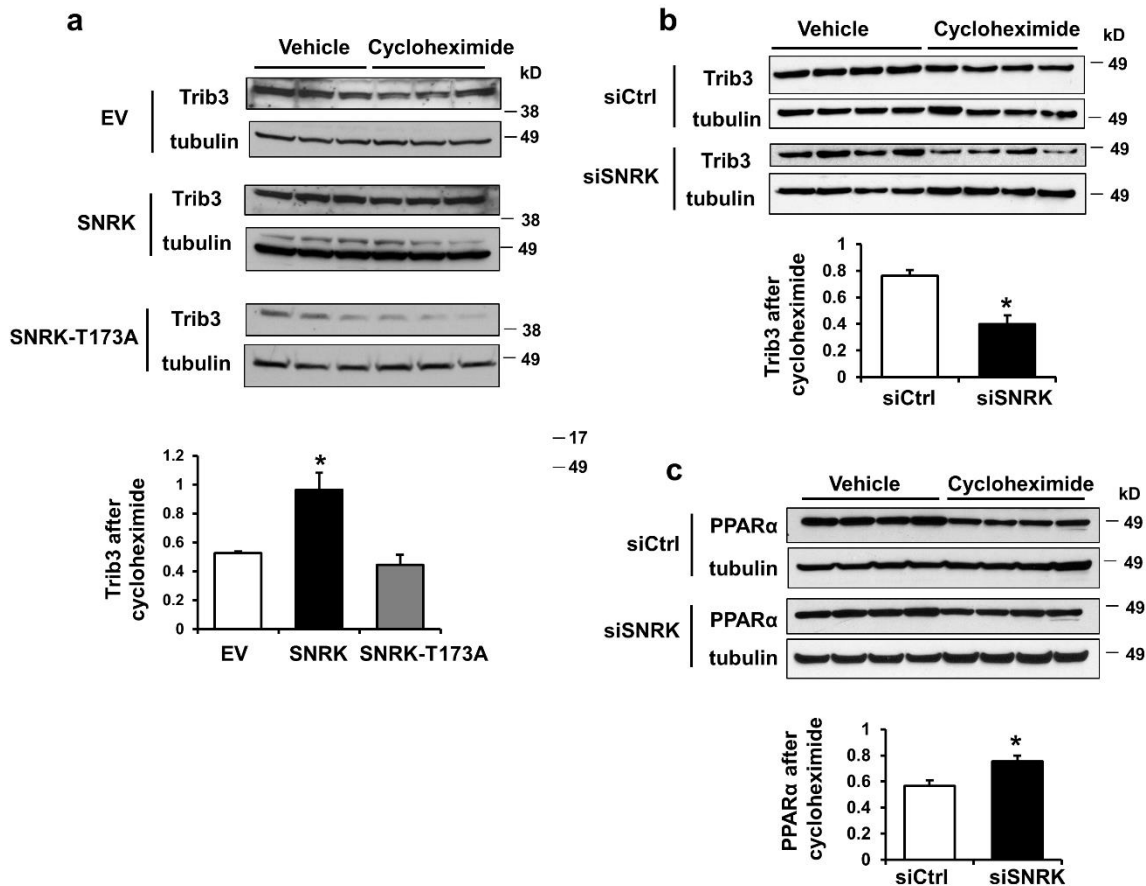
Supplementary Figure 4: Mitochondrial complex activity, content, and ultrastructure are not altered by SNRK. (a) Mitochondrial complex I, II, and IV activity in NTG and SNRK TG hearts. n=6. (b) Mitochondrial DNA content in NTG and SNRK TG mouse hearts (n=6), and WT and *Snrk*^{+/-} hearts (n=3). (c) Mitochondrial DNA content in HL1 cells with GFP or SNRK overexpression, or control or SNRK siRNA treatment. n=3. (d) Representative MitoTracker Green images of mitochondria morphology in HL1 cells with control or SNRK siRNA treatment. Scale bar, 100 μm. (e) Representative electron microscopy images of mitochondrial ultrastructure in NTG and SNRK TG hearts, and in WT and SNRK^{+/-} hearts. Scale bar, 1 μm. Data are represented as mean ± SEM.

Supplementary Figure 5



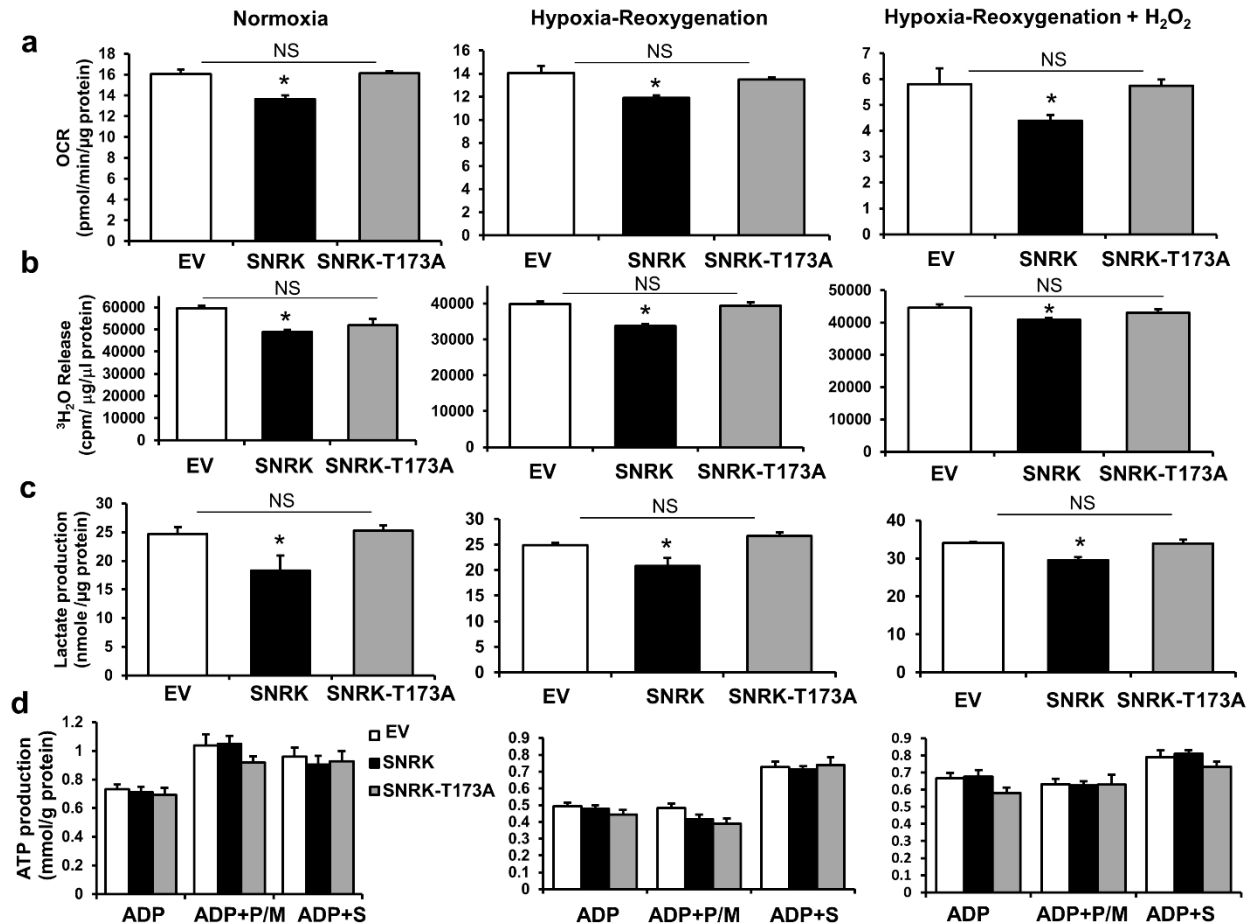
Supplementary Figure 5: Mitochondrial ROS are not altered by SNRK. (a) Mitochondrial ROS in isolated mitochondria from NTG or SNRK TG hearts as measured by Mitosox intensity at baseline and following treatment of mitochondria with Antimycin A (Ant A). n=3. (b) Mitochondrial ROS in isolated mitochondria from WT or *Snrk*^{+/-} hearts. n=3. (c) Mitochondrial ROS in HL1 cells with GFP or SNRK overexpression. n=3. (d) Mitochondrial ROS in HL1 cells with control (siCtrl) or SNRK siRNA treatment. n=3. (e) Antioxidant gene expression in NTG and SNRK TG hearts. SOD = superoxide dismutase, Cat = catalase, Gpx = glutathione peroxidase, Prdx = peroxiredoxin. n=3. (f) SOD2 expression in NTG and SNRK TG hearts. (g) SOD2 expression in HL1 cardiomyocytes with empty vector (EV) or SNRK overexpression. Data are represented as mean ± SEM. **P*≤0.05 by Student's t-test.

Supplementary Figure 6



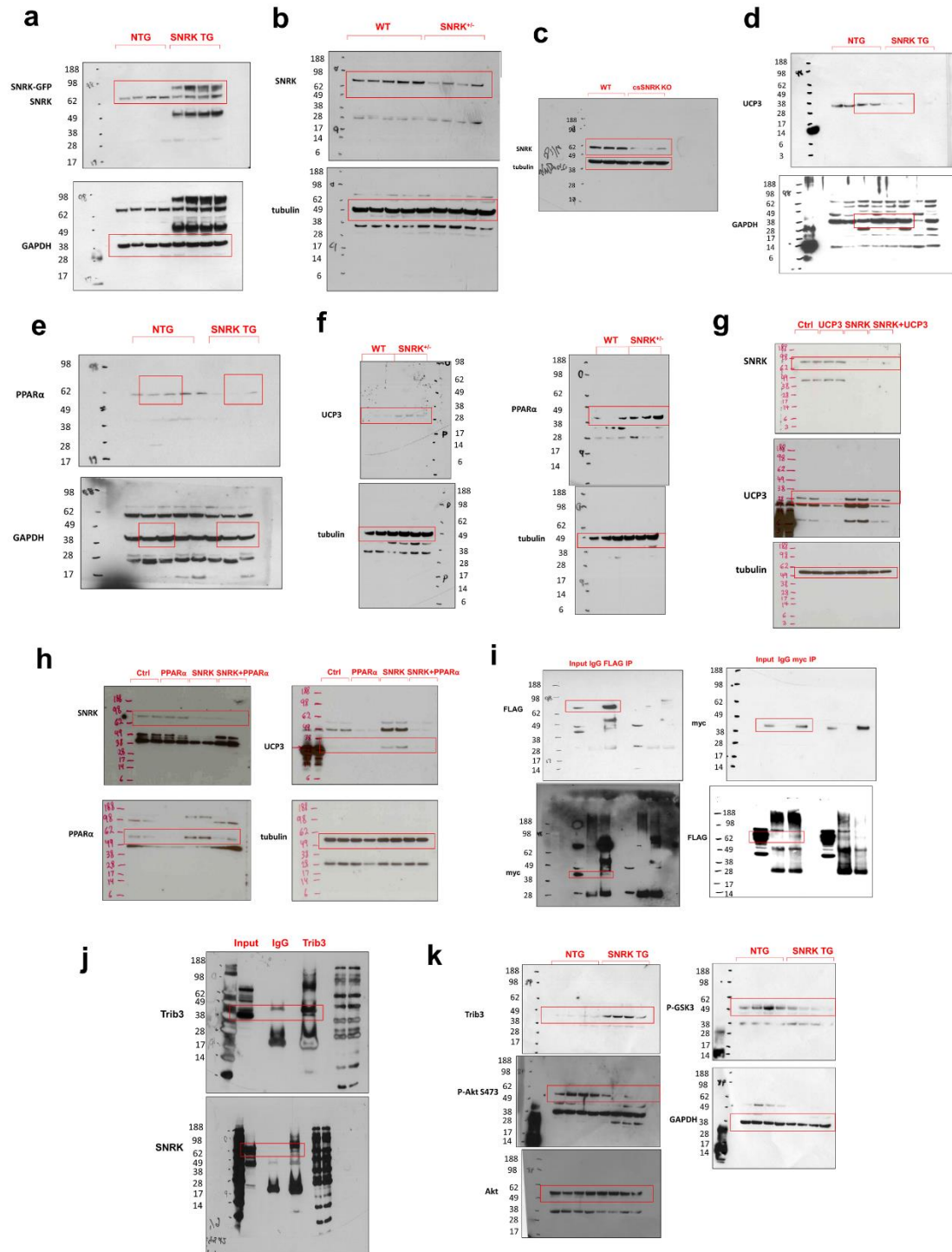
Supplementary Figure 6: SNRK increases protein stability of Trib3 and decreases stability of PPARα. (a) Trib3 protein levels in HL1 cardiomyocytes with empty vector, SNRK, or SNRK-T173A overexpression without and with 4 hours of 150 μg/mL cycloheximide treatment. (b) Trib3 protein levels in HL1 cardiomyocytes with control or SNRK siRNA treatment without and with cycloheximide treatment. (c) PPARα protein levels in HL1 cardiomyocytes with control or SNRK siRNA treatment without and with cycloheximide treatment. Data are represented as mean ± SEM. * $P \leq 0.05$ by one-way ANOVA or Student's t-test.

Supplementary Figure 7



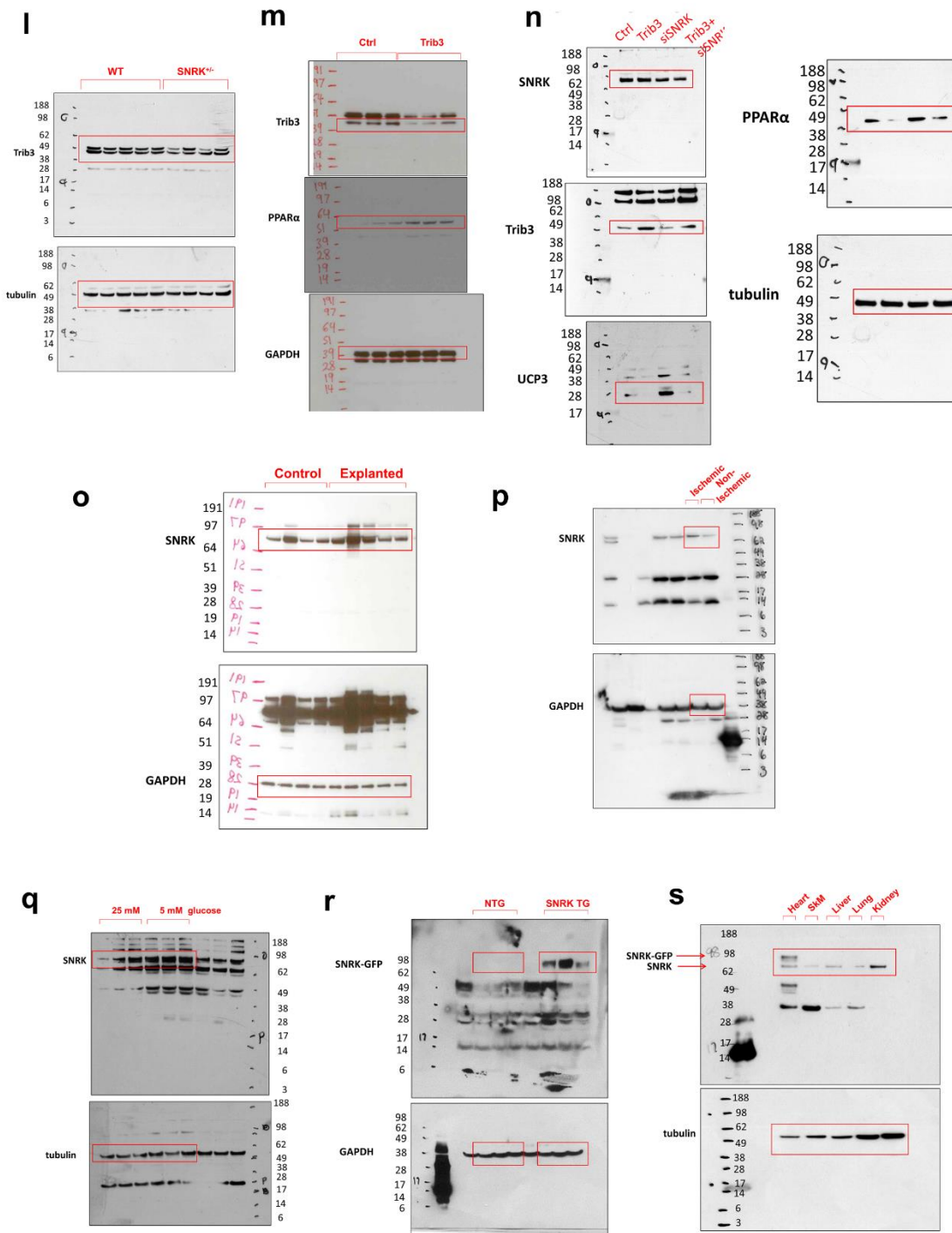
Supplementary Figure 7: SNRK decreases oxygen consumption and metabolic flux during hypoxia-reoxygenation. (a) Oxygen consumption in HL1 cells with empty vector (EV) control, SNRK, or SNRK-T173A overexpression in the presence of normoxia (n=29-32), hypoxia-reoxygenation (n=14-16), or hypoxia-reoxygenation with H₂O₂ treatment (n=14-16). (b) Palmitate oxidation in HL1 cells with control, SNRK, or SNRK-T173A overexpression in the presence of normoxia, hypoxia-reoxygenation, or hypoxia-reoxygenation with H₂O₂ treatment. n=3-5. (c) Lactate production in HL1 cells with control, SNRK, or SNRK-T173A overexpression in the presence of normoxia (n=3), hypoxia-reoxygenation (n=4-6), or hypoxia-reoxygenation with H₂O₂ treatment (n=3-4). (d) ATP production in HL1 cells with control, SNRK, or SNRK-T173A overexpression in the presence of normoxia, hypoxia-reoxygenation, or hypoxia-reoxygenation with H₂O₂ treatment. n=11-14. P/M = pyruvate/malate, S = succinate. n_≥3. Data are represented as mean ± SEM. *P≤0.05 by Student's t-test.

Supplementary Figure 8



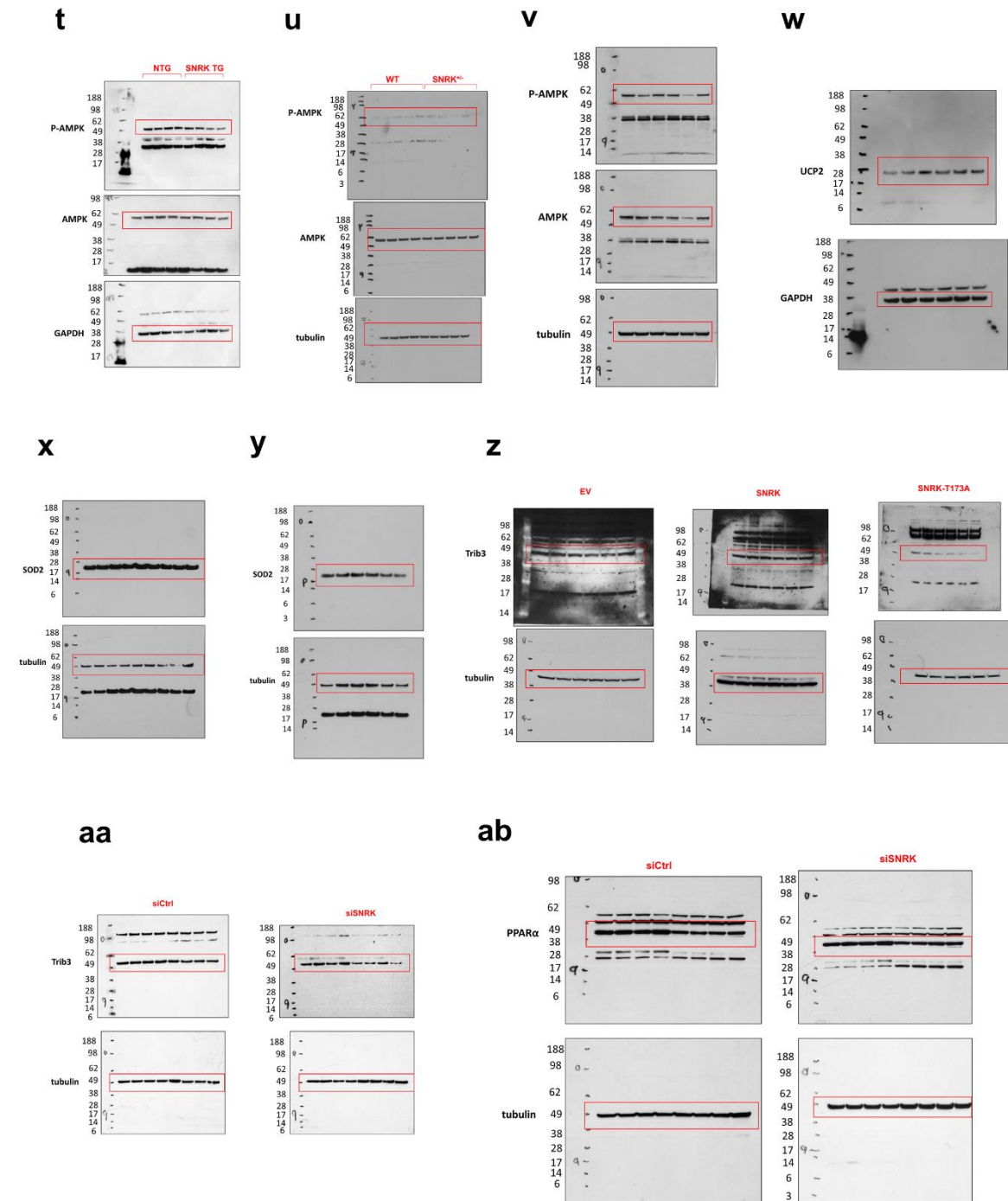
Supplementary Figure 8: Unedited Western blots. Uncut blots for (a) Fig. 1a, (b) Fig. 1g, (c) Fig. 1j, (d) Fig. 2e, (e) Fig. 2e, (f) Fig. 2g, (g) Fig. 3a, (h) Fig. 3f, (i) Fig. 4a, (j) Fig. 4b, and (k) Fig. 4d.

Supplementary Figure 8



Supplementary Figure 8: Unedited Western blots. Uncut blots for **(l)** Fig. 4e, **(m)** Fig. 4f, **(n)** Fig. 4g, **(o)** Fig. 5a, **(p)** Fig. 5b, **(q)** Fig. 5c, **(r)** Supplemental Fig. 1a, and **(s)** Supplemental Fig. 1b.

Supplementary Figure 8



Supplementary Figure 8: Unedited Western blots. Uncut blots for (t) Supplemental Fig. 2a, (u) Supplemental Fig. 2b, (v) Supplemental Fig. 2e, (w) Supplemental Fig. 2f, (x) Supplemental Fig. 4f, (y) Supplemental Fig. 4g, (z) Supplemental Fig. 6a, (aa) Supplemental Fig. 6b, and (ab) Supplemental Fig. 6c.

Supplementary Table 1

	Body weight (g)	Wet heart weight (mg)	Dry heart weight (mg)	Heart rate (beats/min)	Aortic SP (mm Hg)
NTG	22.9±0.43	136±3.48	26.8±0.90	291±6.98	79.9±1.32
TG	22.3±0.52	136±4.24	26.6±0.92	286±8.34	79.4±0.97

	Aortic DP (mm Hg)	Aortic DevP (mm Hg)	Cardiac flow (L/min/m²)	Aortic flow (L/min/m²)	Cardiac output (mL/min)
NTG	40.8±1.85	39.2±1.96	2.90±0.08	8.53±0.26	11.4±0.29
TG	41.8±1.06	37.6±1.15	2.96±0.13	8.57±0.21	11.5±0.25

Supplementary Table 1: Body weight, heart weight, and cardiac functional measurements of mice and hearts used in SNRK TG isolated working heart perfusions. SP = systolic pressure; DP = diastolic pressure; DevP = developed pressure. n=12.

Supplementary Table 2

	IVS;d (mm)	LVID;d (mm)	LVPW;d (mm)	IVS;s (mm)	LVID;s (mm)	LVPW;s (mm)	LV Vol;d (μ L)	LV Vol;s (μ L)
NTG	0.76 $\pm$ 0.02	3.84 $\pm$ 0.08	0.67 $\pm$ 0.04	1.16 $\pm$ 0.03	2.73 $\pm$ 0.09	1.00 $\pm$ 0.05	64.5 $\pm$ 3.26	29.0 $\pm$ 2.40
TG	0.76 $\pm$ 0.02	3.72 $\pm$ 0.07	0.74 $\pm$ 0.04	1.12 $\pm$ 0.03	2.52 $\pm$ 0.08	1.09 $\pm$ 0.05	59.3 $\pm$ 2.53	23.4 $\pm$ 1.86

Supplementary Table 2: Cardiac functional measurements in NTG and SNRK TG mice.

IVS = interventricular septum thickness; LVID = left ventricular internal dimension; LVPW = left ventricular posterior wall thickness; LV Vol = left ventricular volume; d = diastolic; s = systolic. n=16-18.

Supplementary Table 3

	Body weight (g)	Wet heart weight (mg)	Dry heart weight (mg)	Heart rate (beats/min)	Aortic SP (mm Hg)	Aortic DevP (mm Hg)
WT	29.9±1.1	161±10	48.5±2.8	318±17.8	72.7±1.01	17.8±1.00
<i>Snrk</i>^{+/-}	29.6±0.78	155±4.1	50.2±2.7	279±18.9	73.3±1.17	15.9±2.34

Supplementary Table 3: Body weight, heart weight, and cardiac functional measurements of mice and hearts used in *Snrk*^{+/-} isolated working heart perfusions. SP = systolic pressure; DevP = developed pressure. n=4-5.

Supplementary Table 4

	IVS;d (mm)	LVID;d (mm)	LVPW;d (mm)	IVS;s (mm)	LVID;s (mm)	LVPW;s (mm)	LV Vol;d (μ L)	LV Vol;s (μ L)
WT	0.68 $\pm$ 0.02	3.62 $\pm$ 0.16	0.55 $\pm$ 0.03	0.84 $\pm$ 0.03	2.70 $\pm$ 0.24	0.76 $\pm$ 0.08	58.7 $\pm$ 6.17	24.7 $\pm$ 3.18
<i>Snrk</i>^{+/-}	0.66 $\pm$ 0.05	3.62 $\pm$ 0.01	0.53 $\pm$ 0.16	0.84 $\pm$ 0.06	2.76 $\pm$ 0.09	0.94 $\pm$ 0.08	54.3 $\pm$ 2.33	21.0 $\pm$ 1.00

Supplementary Table 4: Cardiac functional measurements in WT and *Snrk*^{+/-} mice. IVS = interventricular septum thickness; LVID = left ventricular internal dimension; LVPW = left ventricular posterior wall thickness; LV Vol = left ventricular volume. n=3.

Supplementary Table 5

	IVS;d (mm)	LVID;d (mm)	LVPW;d (mm)	IVS;s (mm)	LVID;s (mm)	LVPW;s (mm)	LV Vol;d (μl)	LV Vol;s (μl)
WT	0.64 $\pm$ 0.04	3.96 $\pm$ 0.10	0.62 $\pm$ 0.02	1.05 $\pm$ 0.04	2.67 $\pm$ 0.09	0.99 $\pm$ 0.05	26.96 $\pm$ 1.80	68.34 $\pm$ 3.23
<i>csSnrk</i> KO	0.73 $\pm$ 0.04	3.84 $\pm$ 0.13	0.67 $\pm$ 0.03	1.09 $\pm$ 0.07	2.57 $\pm$ 0.10	1.04 $\pm$ 0.03	26.33 $\pm$ 2.41	67.38 $\pm$ 5.44

Supplementary Table 5: Cardiac functional measurements in WT and *csSnrk* KO mice.

IVS = interventricular septum thickness; LVID = left ventricular internal dimension; LVPW = left ventricular posterior wall thickness; LV Vol = left ventricular volume. n=6-10.

Supplementary Table 6

	Body weight (g)	Wet heart weight (mg)	Dry heart weight (mg)	Heart rate (beats/min)	Aortic SP (mm Hg)	Aortic DevP (mm Hg)
WT	25.72±0.45	144.1±3.9	34.7±1.0	294±15.6	71.0±1.3	24.5±1.1
csSnrk KO	24.87±0.80	142.6±2.9	34±1.1	293±11	68.7±1.2*	18.3±1.4*

Supplementary Table 6: Body weight, heart weight, and cardiac functional measurements of mice and hearts used in csSnrk KO isolated working heart perfusions. SP = systolic pressure; DevP = developed pressure. n=6 for WT, n=10 for csSnrk KO. * $P < 0.05$ by Student's t-test.